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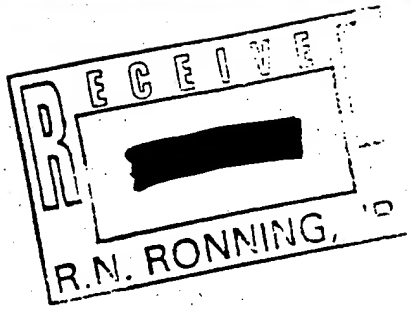
Memorandum

TO: PETER THOMPSON
NOEL WARNER
ROY RONNING

FROM: LEON TERSTAPPEN

DATE: [redacted]



RE: PHAGE DISPLAY (ROY HAS THIS INFORMATION ALREADY)
EXPLANATION/RESULTS/AND POTENTIAL PATENTABLE ITEMS

Enclosed:

I. 8 pages explaining phage display and in particularly how Ton's library is created. Note that the library is made such that it selects for functional antibodies (page 7).

II. 13 pages showing how phage mab's which bind cell subsets were selected. The sort criteria used for selection. The results of the first exploratory experiments. Pay close attention to page 3, 4, 5 and 6, since these data are also applicable to Nexamer's technology.
Page 6 through 12 shows the actual staining profile of the binders.
Can we or should we patent these scFv antibodies:

A1 / E1 / E2 / T1 / T2 / B14 / B28

III. 5 pages each of which describes a potential application of this technology. Note these are ideas generated by Ton and myself and maybe already thought of by many others.

IV. 1 page which shows the generation of scFv antibodies to various substances. The importance is that phages with different CDR3 sequences are found which bind to the same substrate. Does this circumvent claims in other patents, i.e. a polyclonal approach?

V. Once one or more phages are identified which specifically identify a target substrate (including cells) strategies can be followed which permit visualization of the phage.

- generate single chain Fv and genetically engineer a tag onto the scFv (page V1)

- leave the phage scFv intact and use the phage as the detection vehicle

  - antibodies directed against the phage (used in I page 6 → 12)
  - fluorescently label the phage for instance the proteins
  - introduce genes which encoded for detectable probes or linkers. (cysteines, streptags, luciferase)
  - use the density of the phage to separate bound from unbound. (QBC applications)

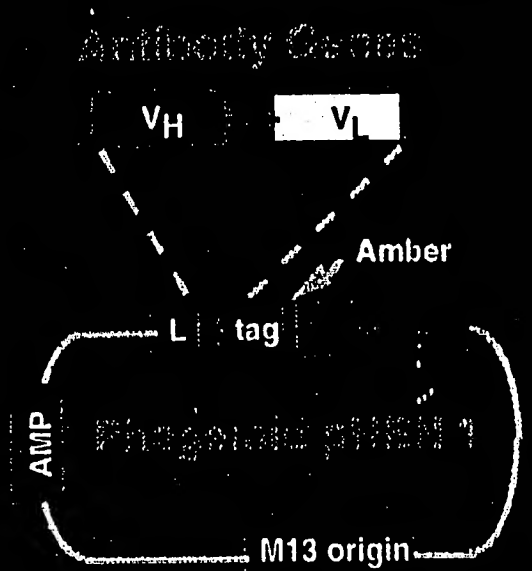
Phage Display

Expression of peptides / proteins as fusion proteins on the surface of filamentous bacteriophages

I

2

Phagemid display and secretion



suppressor
& helper phage

Phage Antibody

non-suppressor

Single chain Fv

Phage Antibody Libraries

Phage antibody libraries contain millions of different antibody molecules expressed on the phages

The optimal optimal library contains high affinity antibodies of every conceivable specificity

Phage Antibody Display

Expression of fragments of antibody molecules (scFv / Fab) on the surface of filamentous bacteriophages

Generation of a Phage Library of human scFv antibodies

- Use 50 germline VH gene segments
- Generate synthetic CDR3 regions of variable length (6-15 amino acid residues)
- Randomise stretches of amino acids in the CDR3 region

I

6

Generation of a Large Phage Library of Human scFv antibodies

- Introduction of seven different light chain V regions (both κ , λ)

- Combination of all sublibraries to a single "master library" of 3.8×10^8 specificities

Variability in V_H - CDR3 sequences

CR6	
CR9A	A FDS D Y G
CR9B	FFDS S Y Y
CR12A	SSGFDY D YYW G
CR12B	SGFDS Y YW Y
CR14	D ANSNSGFDY Y GSYYYW ST WY
CR15A	D NANASNSGFDY Y SGSGYYYW TSTS YWYW
CR15B	DNANF SNSGFDY YSGSS YYYW TSTY YWY

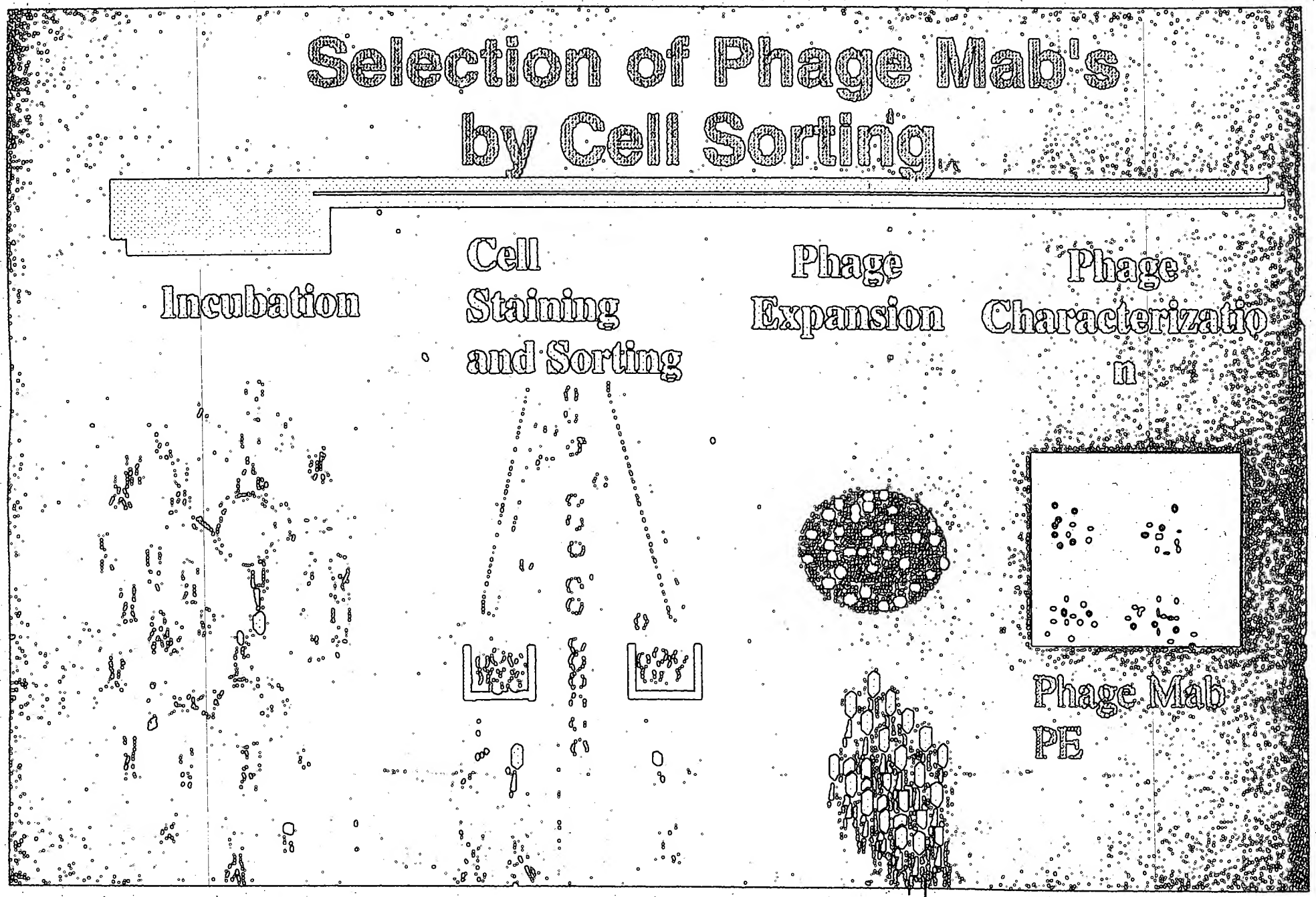
Phage Antibody Display

Biomolecular interactions with the target structure allows the selection of specific phage antibodies

II

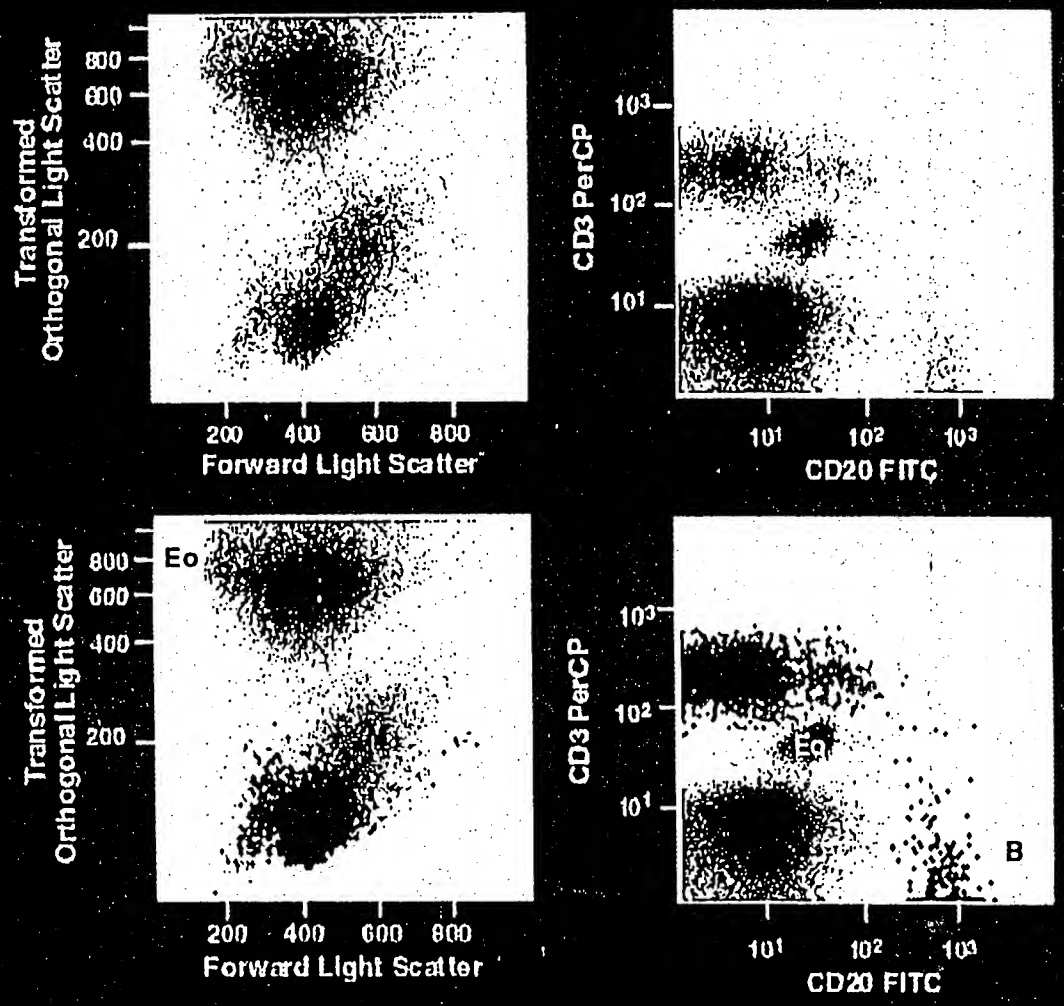
(1)

Selection of Phage Mab's by Cell Sorting



II

Sort criteria used to select leukocytes, eosinophils, B-lymphocytes and T-lymphocytes



II

(3)

Phages recovered from increasing numbers of sorted blood cells

Cell Population		Number of sorted cells: 10 #phages	100 #phages	1,000 #phages	10,000 #phages
(100%)	Exp 1	0	5	48	640
	Exp 2	0	0	14	250
(8%)	Exp 1	2	9	174	1,280
(20%)	Exp 1	2	1	41	320
	Exp 2	0	4	22	100
B cells (CD20+) (2%)	Exp 1	2	23	444	1,704
	Exp 2	0	29	116	566

10^{13} phages of a phage library (diversity 3.8×10^8) incubated with 5×10^6 peripheral blood leukocytes

Phages recovered from 10,000 sorted blood cells

Cell Population	# Cells incubated with phages: 5 x 10^6 #phages	2.5 x 10^6 #phages	1.2 x 10^6 #phages	0.6 x 10^6 #phages
T cells CD3+	250	780	2,164	3,684
NK cells CD16+	100	2,860	964	8,700
B cells CD20+	566	3,422	6473	11,019

II

5

Phages recovered from 10,000 sorted blood cells

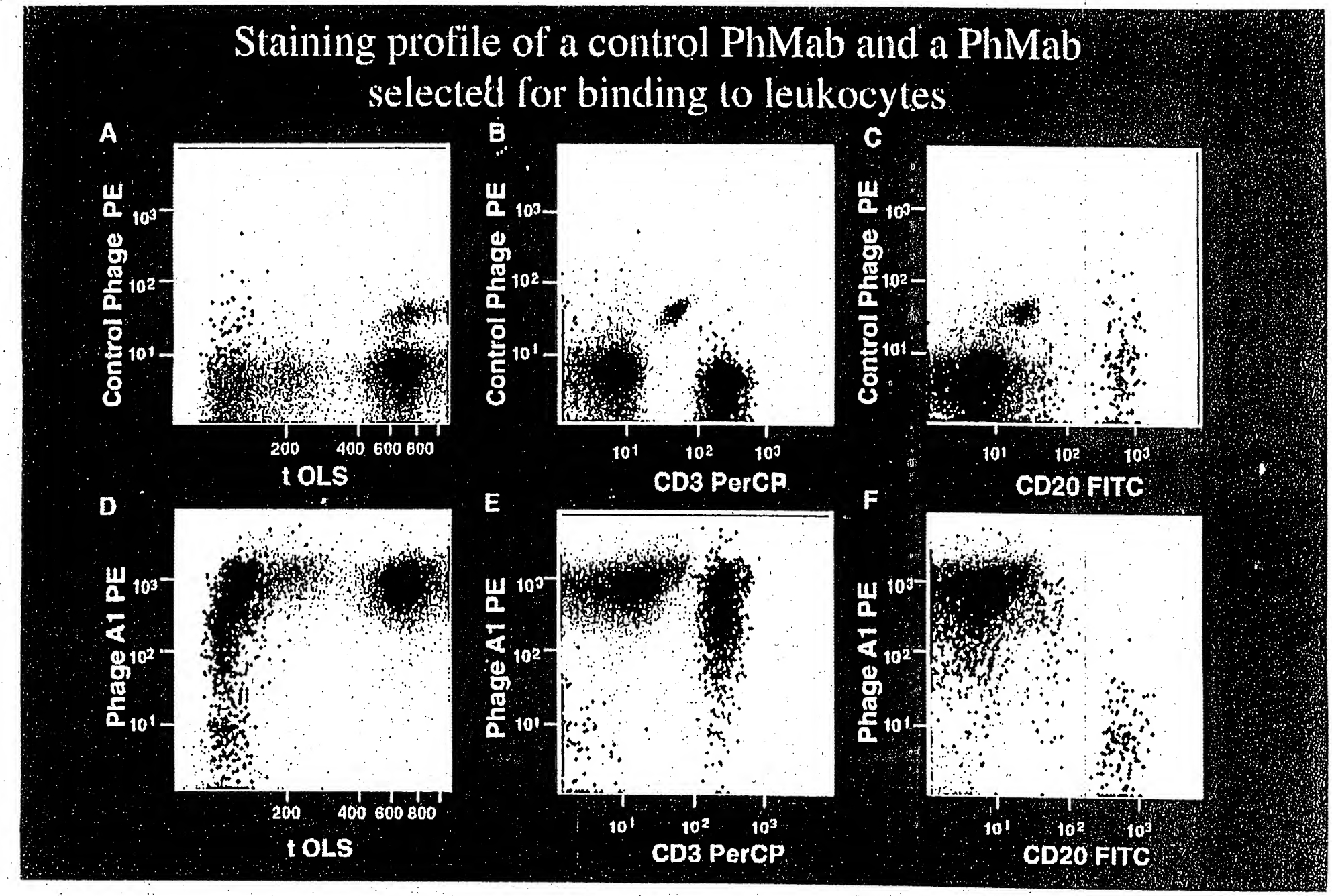
Cell Population		1st Selection #phages	2nd Selection #phages
[illegible]	Exp 1	640	980
[illegible]	Exp 1	1,280	390
[illegible] cells	Exp 1	320	3,330
	Exp 2	200	2,800
[illegible]	Exp 2	260	1,344
[illegible]	Exp 2	180	906
B cells CD20+	Exp 1	1,704	6,000
	Exp 2	500	4,320

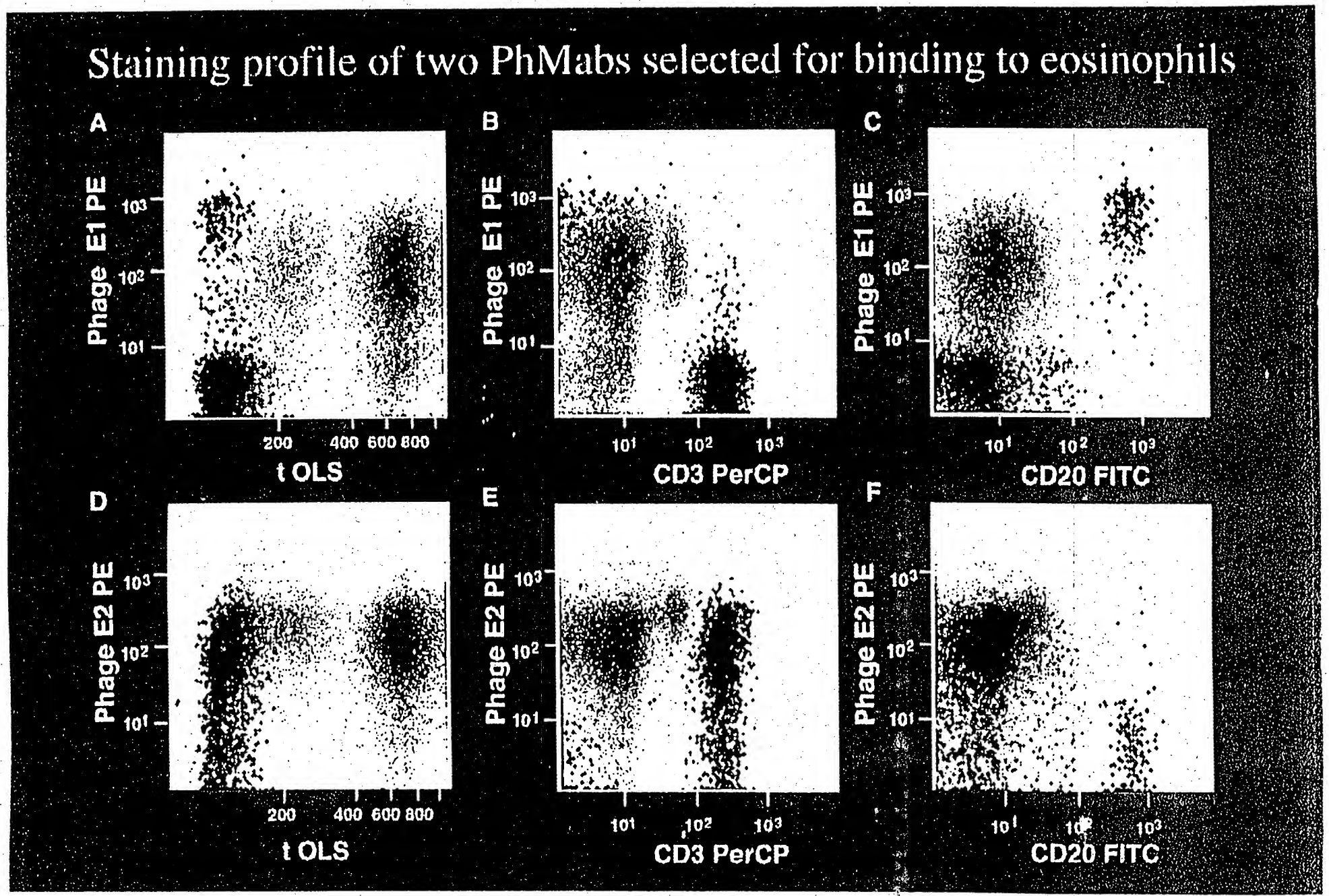
Diversity of phages from sorted blood cells

phages	# phages after 1st Selection	# Mabs tested after 2st Selection	# phages no binding	# phages binding profile 1	# phages binding profile 2
[illegible]	640	15	0	14	1
[illegible]	1,280	15	4	9	3
[illegible]	320	15	0	14	1
B cells CD20+	1,704	16	6	9	1

II

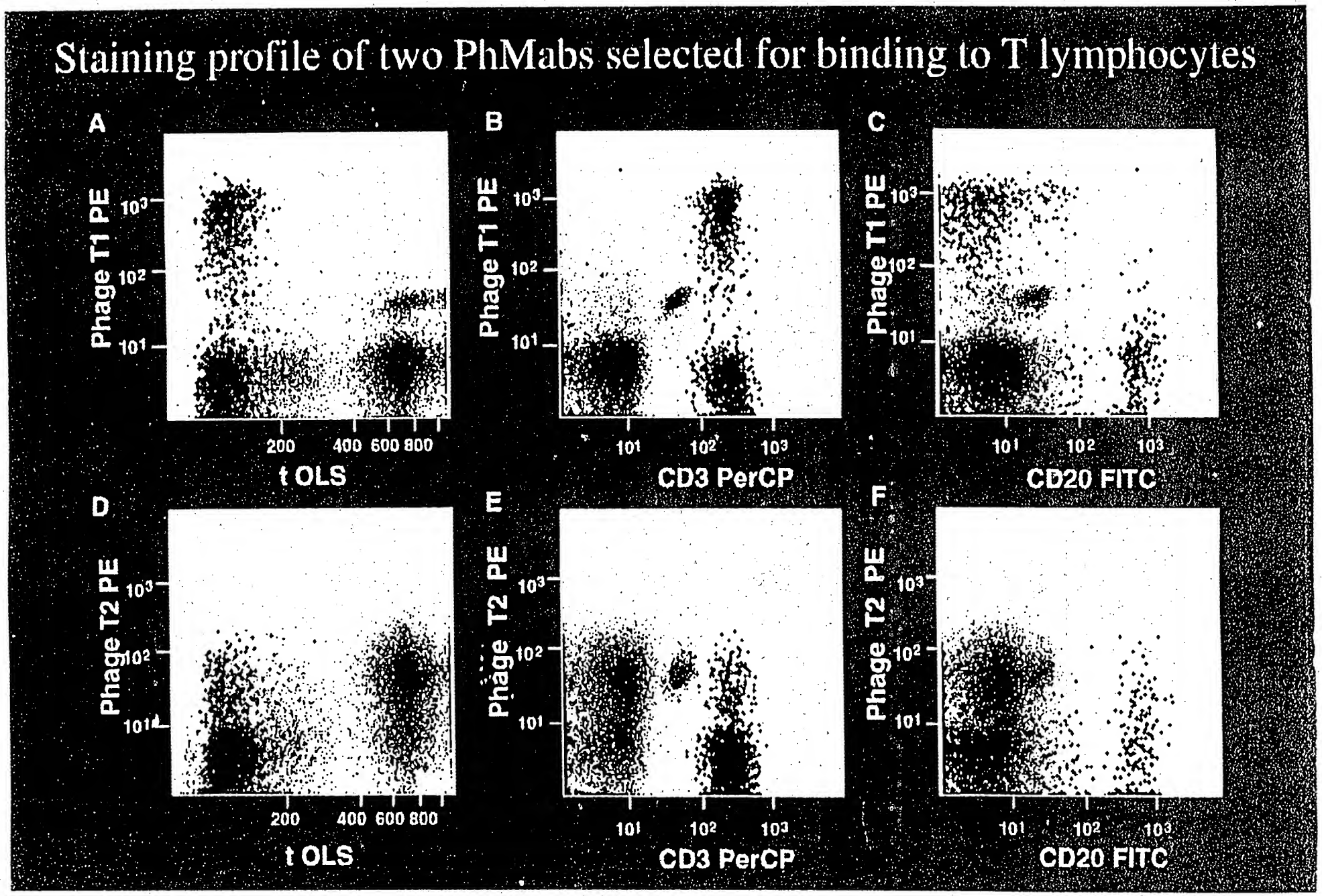
(7)


Staining profile of a control PhMab and a PhMab selected for binding to leukocytes
A
B
C
D
E
F
Control Phage PE
Phage A1 PE
10¹
10²
10³
200
400
600
800
t OLS
CD3 PerCP
CD20 FITC

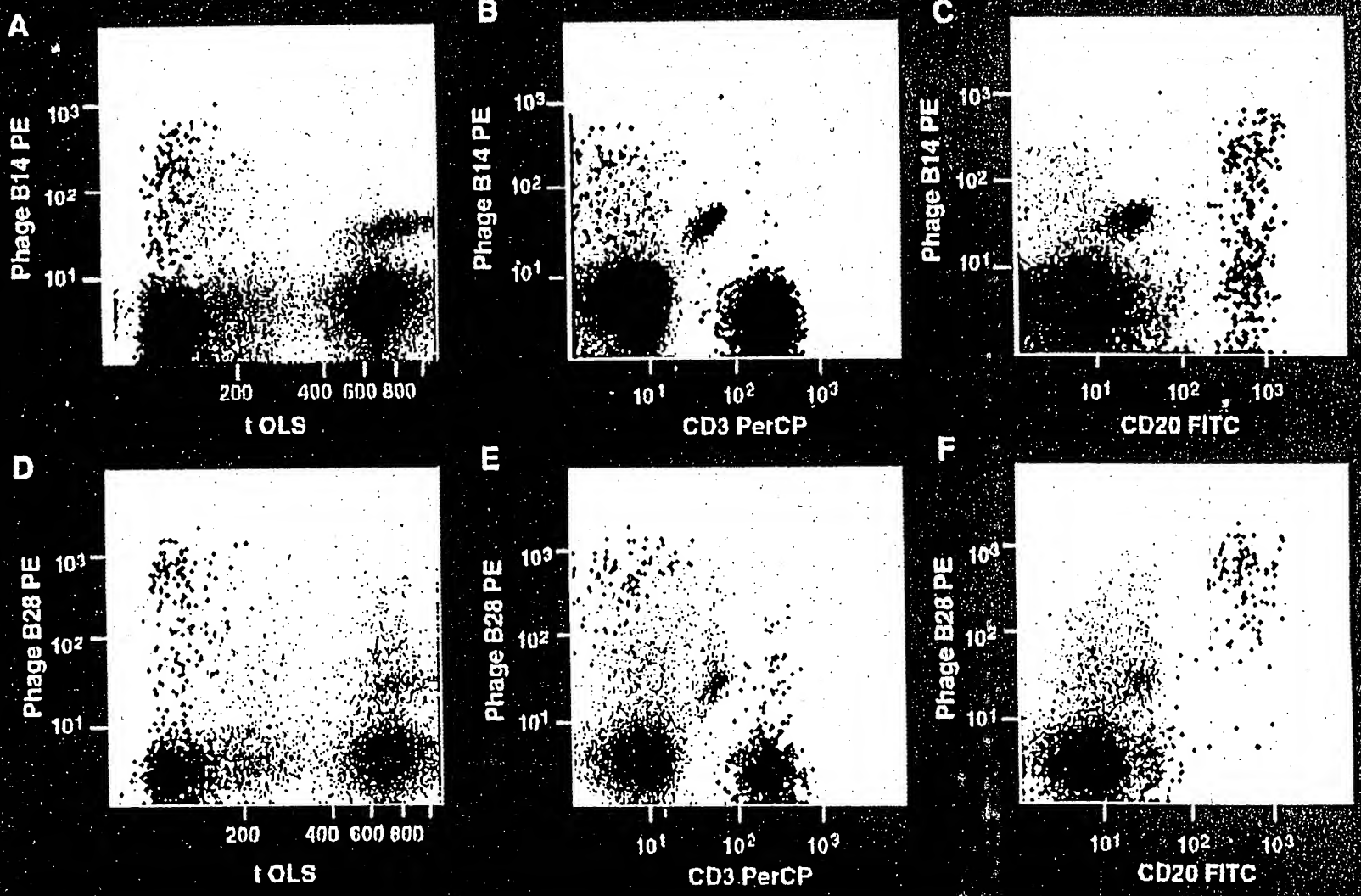

Staining profile of two PhMabs selected for binding to eosinophils
A
B
C
D
E
F
Phage E1 PE
Phage E2 PE
10^1
10^2
10^3
200
400
600
800
t OLS
CD3 PerCP
CD20 FITC

II

9


Staining profile of two PhMabs selected for binding to T lymphocytes
A
B
C
D
E
F
Phage T1 PE
Phage T2 PE
10³
10²
10¹
200
400
600
800
t OLS
CD3 PerCP
CD20 FITC

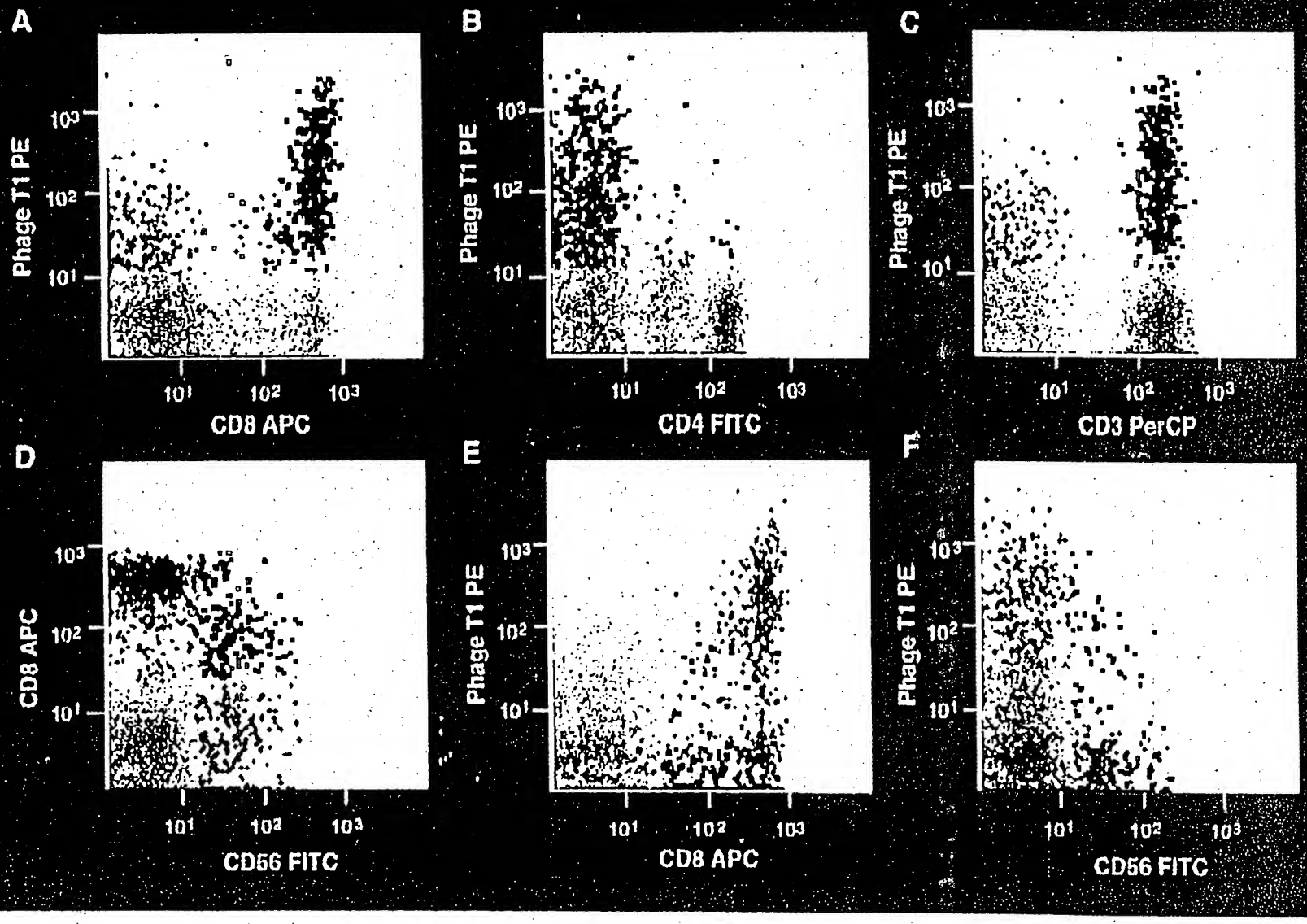
Staining profile of two PhMabs selected for binding to B-Lymphocytes



II

11

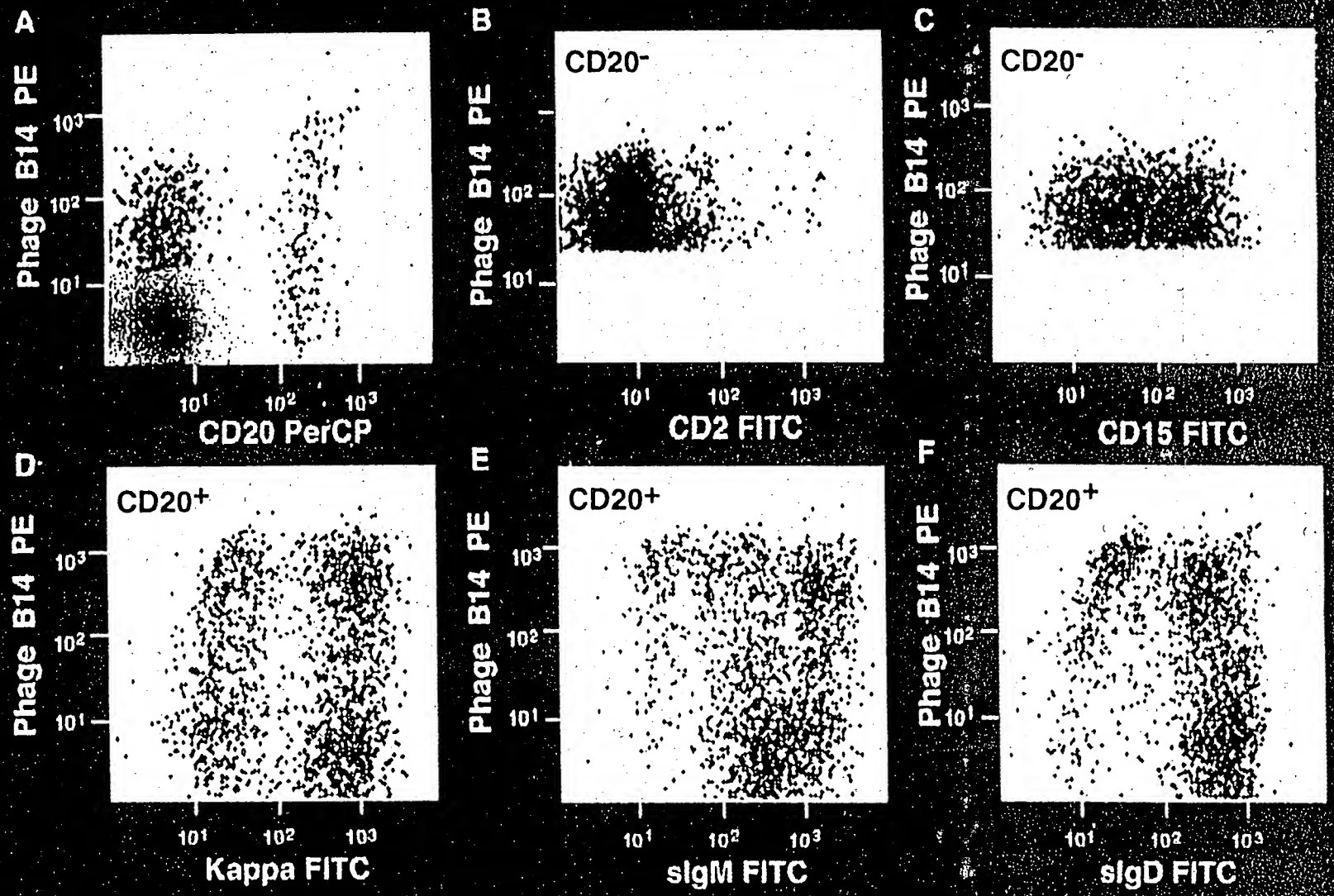
Characterization of PhMab T1 selected for binding to T lymphocytes



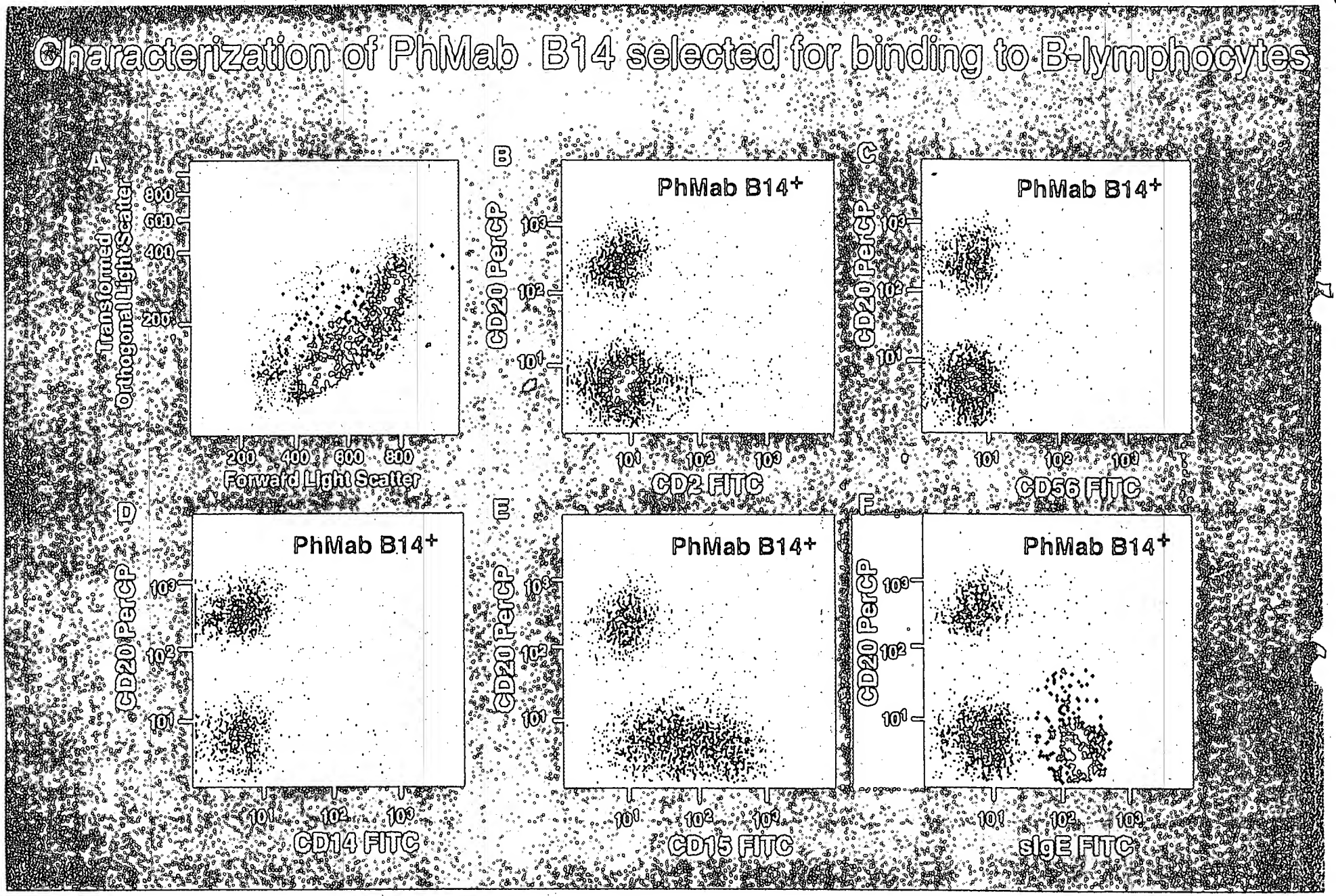
II

(12)

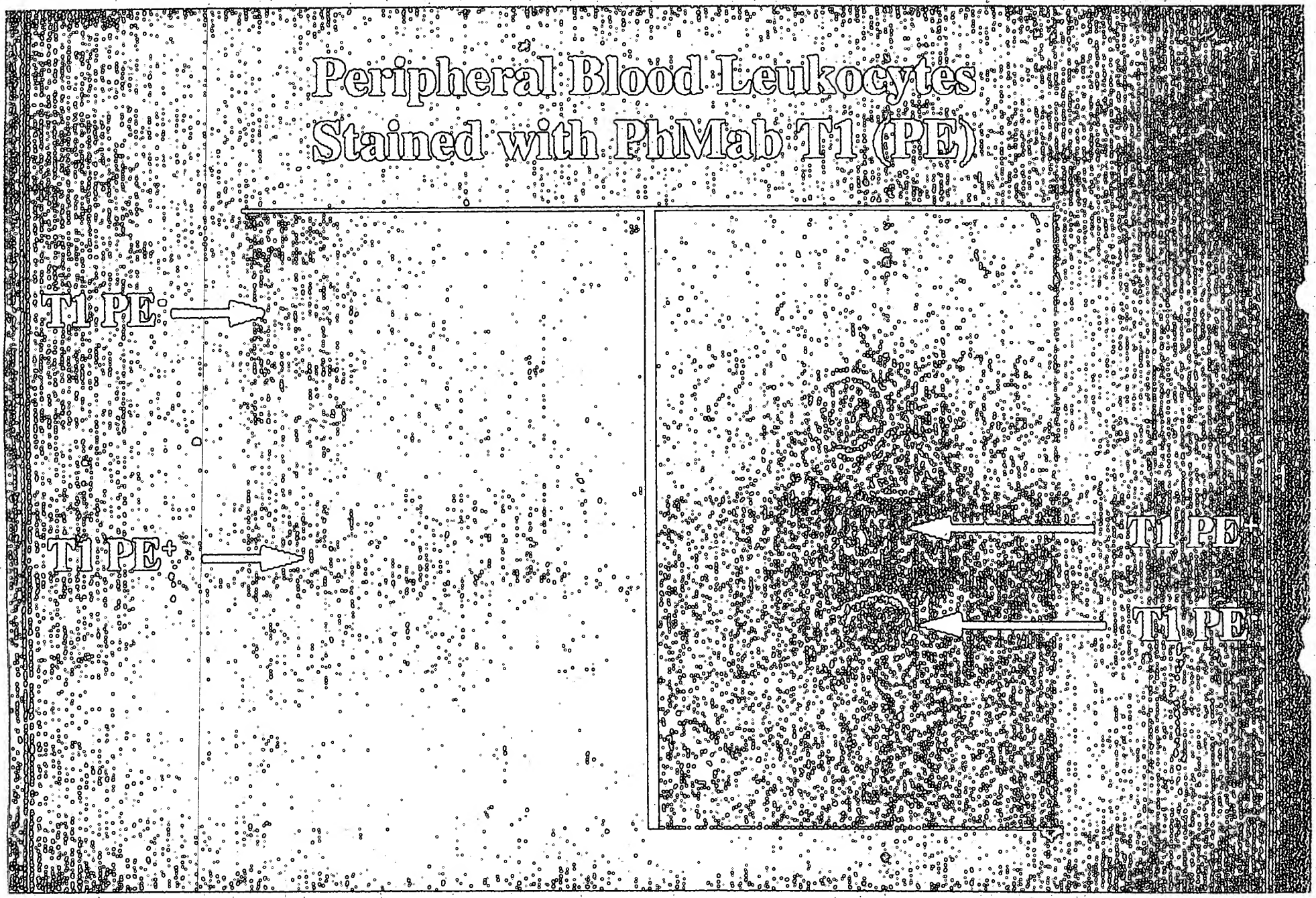
Characterization of PhMab B14 selected for binding to B-lymphocytes



Characterization of PhMab B14 selected for binding to B-lymphocytes



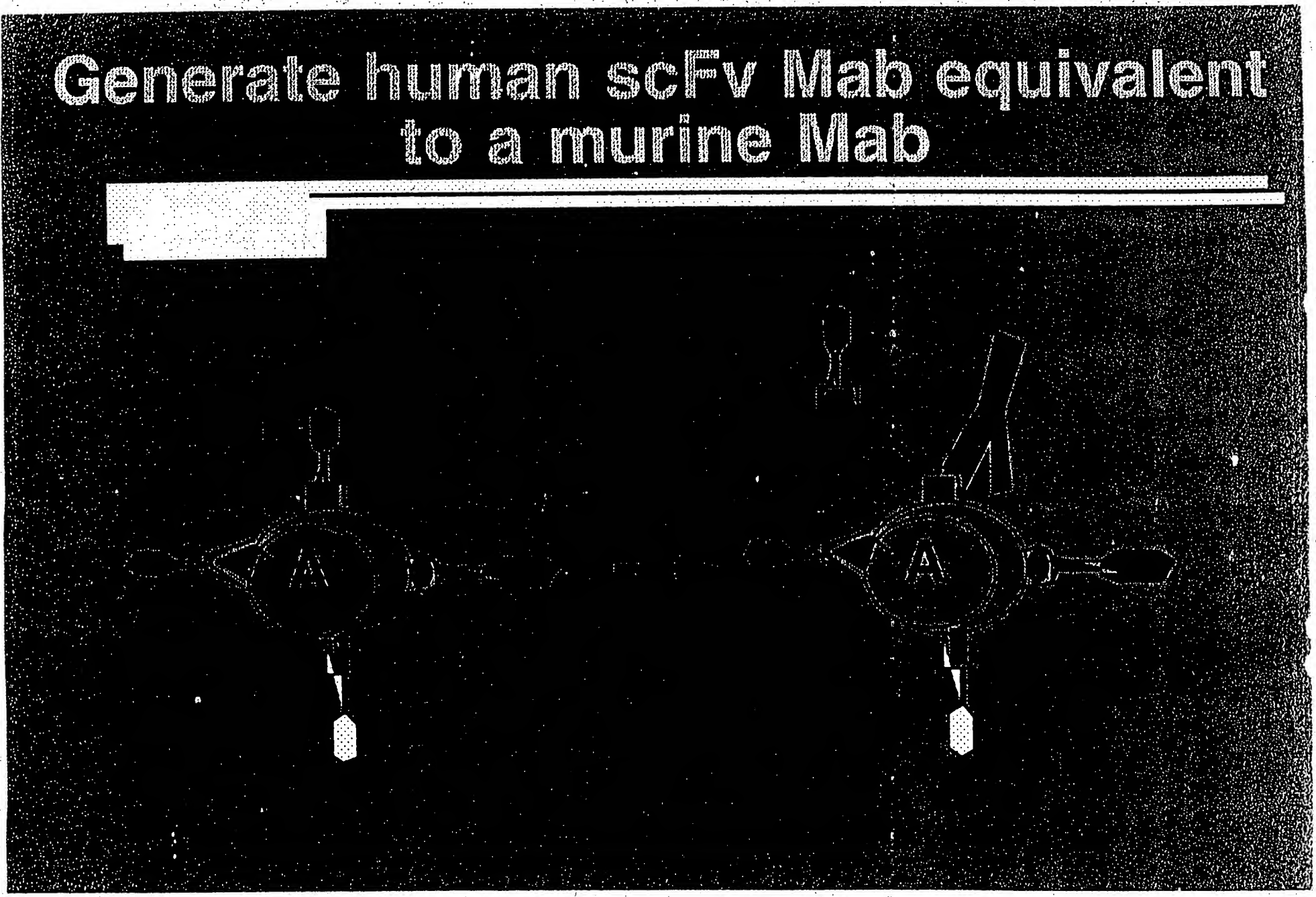
II


Peripheral Blood Leukocytes
Stained with PhMab T1 (PE)
T1 PE-
T1 PE+
T1 PE+
T1 PE-

III

(1)

Generate human scFv Mab equivalent to a murine Mab



III

(2)

Identification of cell specific human scFv Mab's

'A' binders

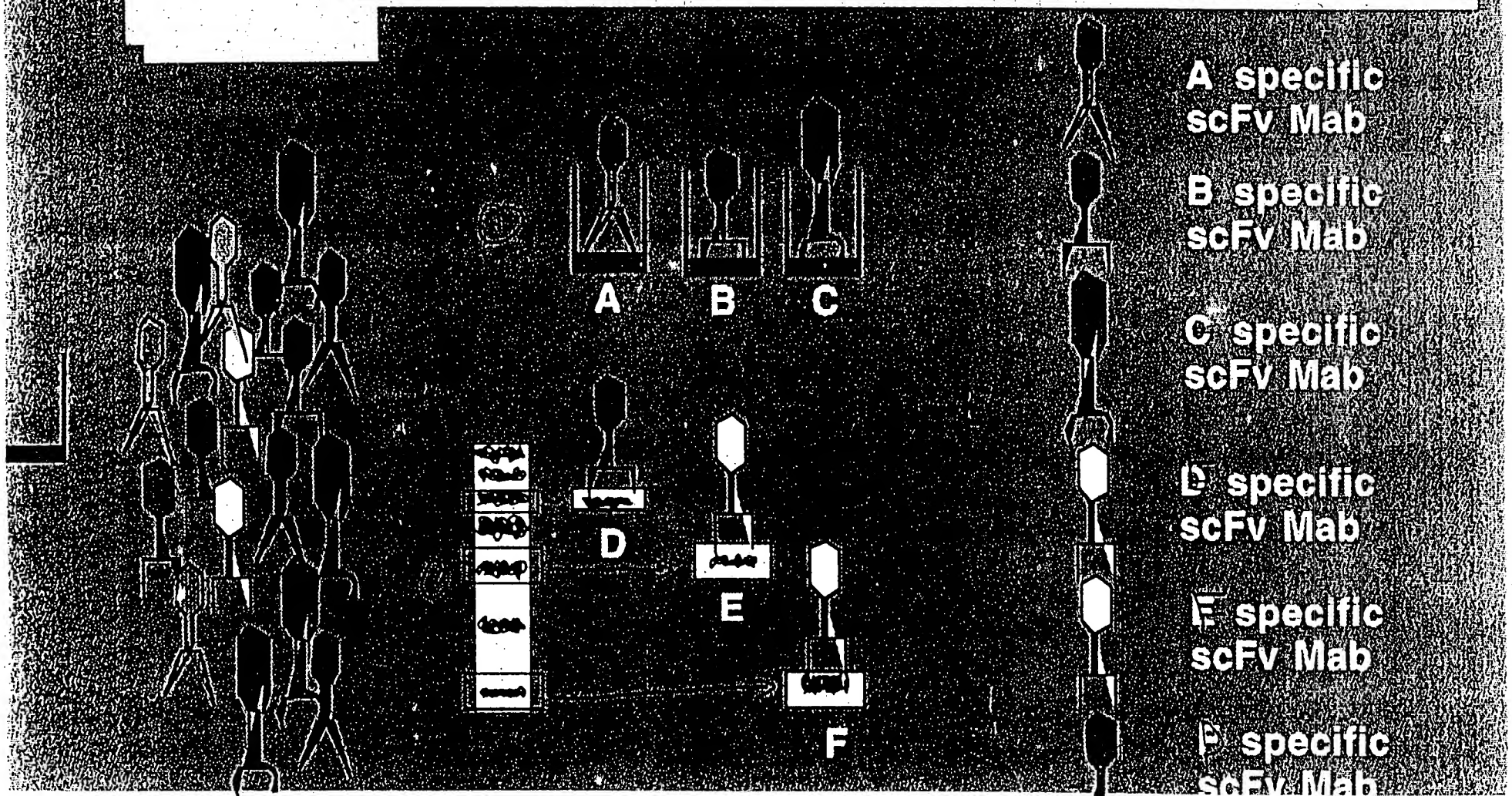
'B' binders

'A'only binder

III

(3)

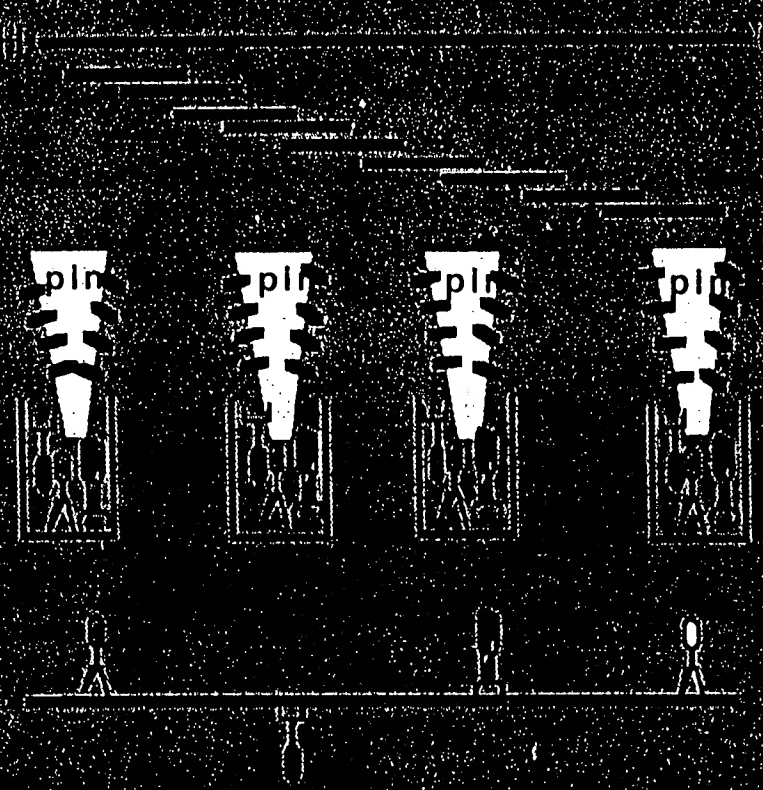
Generation of Substrate Specific Single Chain Antibodies



III

(4)

Selection of Epitope Specific scFV by Pepscan



Overlapping peptides synthesized on pins

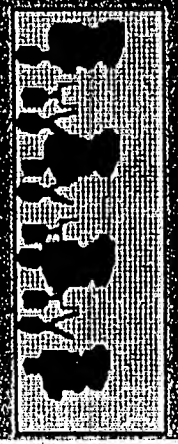
III

5

Identification of Ligands through Receptor Epitope Specific scFV

Receptor
+
Ligand

Receptor



Affinity
Purification

Purified
Ligand